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Passive spatial and temporal integration of excitatory synaptic inputs in cerebellar Purkinje cells of young rats

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Abstract

We have investigated the integration of excitatory (parallel fiber) synaptic inputs in cerebellar Purkinje cells of young rats in vitro and in a compartmental model of such a cell, based on 3D morphological reconstruction. Excitatory synaptic inputs at two independent dendritic sites were activated by electrical stimulation with various delays between the two stimuli. Population postsynaptic potentials summed linearly under current clamp condition when the two dendritic input sites were spatially separated (>200 μm) but sublinearly, in a delay dependent manner, when the input sites were close (<50 μm) to each other. Population postsynaptic currents measured under voltage clamp conditions summed linearly independent of the spatial and temporal separation of inputs. Summation of inputs in a passive compartmental model of a Purkinje cell was similar to that of Purkinje cells in vitro. We show that sublinear summation of neighboring inputs is independent of inhibitory mechanisms and suggest that sublinearity is mainly due to a locally reduced driving force.

Keywords: Dendritic integration; Parallel fiber; Excitation; Timing; Synchrony; Rat

Based on a passive dendritic model Rall [15] predicted a dependence of synaptic summation on both spatial and temporal aspects of synaptic activity arriving on the dendrite. In this model, inputs arriving at neighboring sites would sum sublinearly due to a local reduction in driving force whereas spatially separated inputs would not influence each other and sum linearly. Since then, however, voltage dependent dendritic conductances have been shown to strongly influence summation of synaptic inputs in hippocampal and neocortical pyramidal cells (see reviews in Refs. [8,13,17,19]). We have investigated and compared the spatial and temporal aspects of the summation of parallel fiber input in cerebellar Purkinje cells of young rats (P13–16) in vitro and in passive compartmental models based on anatomical reconstructions of these Purkinje cells.

Details of the experimental procedures have been described elsewhere [1]. In short parasagittal slices (250–300 μm) of cerebellar cortex were prepared from Sprague–Dawley rats (p13–16). Somatic whole-cell recordings were carried out at room temperature (22–26 °C) with a setup similar to that described by Stuart et al. [18]. For voltage clamp recordings pipettes were filled with a solution containing (in mM) 145 CsCl, 1 $MgCl_2$, 5 ethyleneglycol-bis-(β-aminoethylether)-*N,N,N′,N′*-tetra-acetic acid (EGTA), 2 ATP, 10 *N*-2-hydroxyethyl-piperazine-*N′*2-ethanesulphonic acid (HEPES) and 1-4% Lucifer Yellow, pH 7.3. The access resistance of 5–12 MΩ was compensated by 80% using the series resistance compensation of the amplifier (LM/EPC, List, Germany).

Pipette solution for current clamp recordings contained (in mM) 140 K-gluconate, 1 $MgCl_2$, 0.5 $CaCl_2$, 3 NaCl, 5 EGTA, 2 ATP, 5 HEPES and 1-4% Lucifer Yellow, pH 7.3. Current clamp recordings were performed using an Axoclamp-2A amplifier (Axon Instr., USA).

Most Purkinje cells analyzed in this study (20 out of 26) were stained with Lucifer Yellow (Sigma, Germany) and photographed to document the dendritic position of the stimulating electrodes (Fig. 1a,c). Input resistances of Purkinje cells were 187 ± 35 MΩ (current clamp) and

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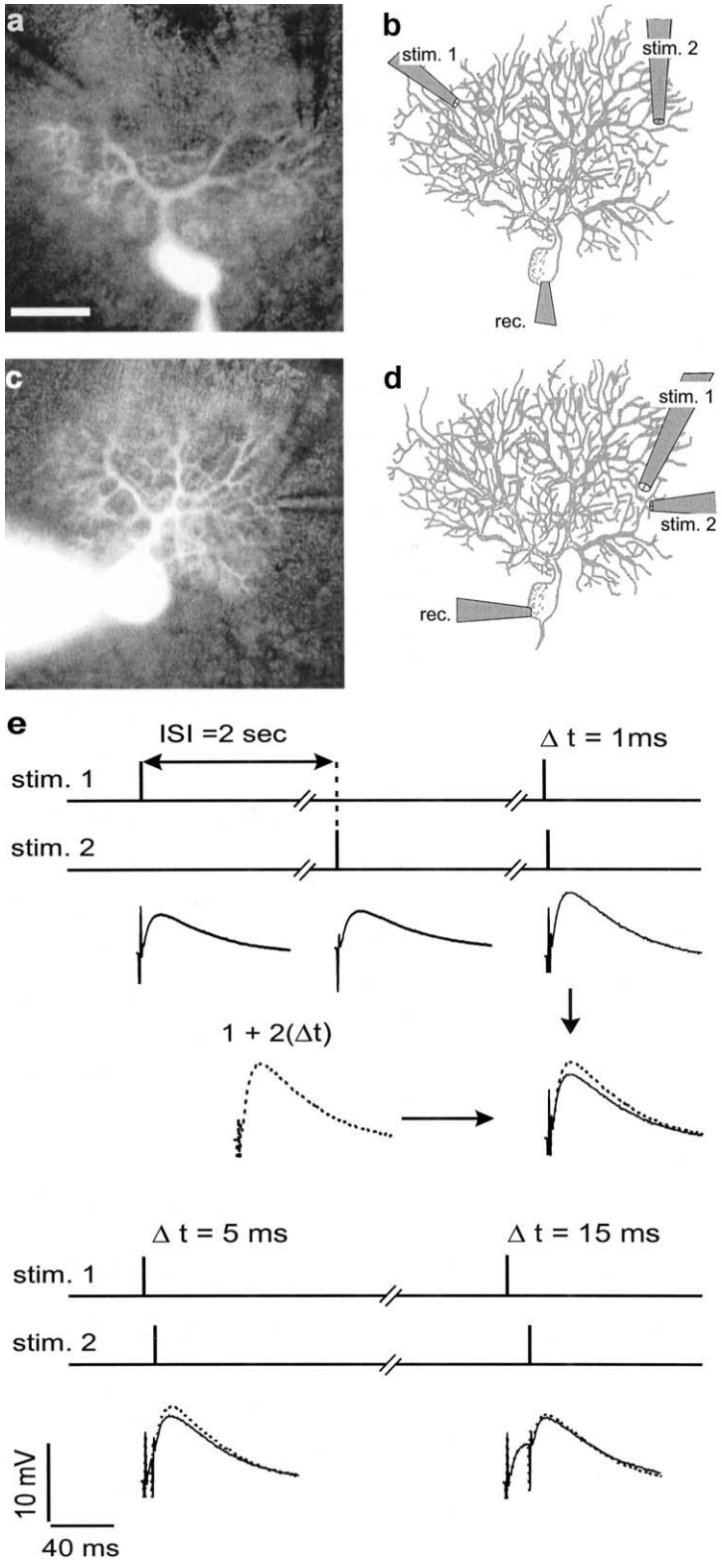

Fig. 1. Spatial arrangement of stimulating electrodes over Purkinje cell dendrites in sagittal slices in vitro and an example of data measured in response to stimulation of neighboring dendritic sites. (a–d) Photograph (Lucifer yellow fluorescence with background illumination) and a schematic drawing of the latter showing electrodes positioned to stimulate distant (a,b) and neighboring (c,d) dendritic sites. Calibration bar in (a) is 50 μm (also valid in (c)). (e) Stimulation protocol (upper two lines: stim1, stim2) and data examples for a stimulation of neighboring dendritic sites. Average responses (solid lines) and calculated average linear prediction (dotted lines) were recorded from the Purkinje cell in (c). Data examples represent average ($n > 20$) responses to individual stimuli and to paired stimuli with delays (Δt) of 1.0, 5.0 and 15.0 ms. The predicted linear response was calculated by point-by-point summation of individual responses with the appropriate time shift ($1 + 2(\Delta t)$, for details see methods section). ISI, inter stimulus interval.

337 ± 112 (SD) MΩ (voltage clamp). In some experiments the $GABA_A$ antagonist (−)-Bicuculline-methiodide and the $GABA_B$ antagonist 2-Hydroxysaclophen (RBI, Germany) were added to the bath medium (50 μM each). Recorded neurons had membrane potentials of at least −60 mV in current clamp experiments and a holding current at a command voltage of −80 mV of less than 100 pA. Somatic voltage and current signals were low-pass filtered at 5 kHz and digitized at 20 kHz (Digitata-1200, Axon Instr., USA).

Bipolar theta-glass electrodes with a tip diameter of ~20 μm were used to electrically stimulate parallel fibers (Fig. 1). Stimulus currents were adjusted to evoke sub-threshold population excitatory postsynaptic potentials (pEPSPs) and currents (pEPSCs) of 2–10 mV or 100–300 pA amplitudes respectively. Amplitude adjustments were the same for distant and neighboring stimulus conditions. In order to quantify the sub-threshold summation of synaptic inputs it was necessary to hyperpolarize the somatic membrane potential to around −80 mV. Effects of voltage dependent mechanisms acting at more depolarized membrane potentials could not be evaluated because at more positive values, stimulation often evoked action potentials and thus made an analysis of synaptic summation impossible. Stimulus currents (0.1 s duration) between 40 and 80 μA, were drawn from two stimulus isolation units (A330, WPI, USA). Inter-stimulus interval was 2 s. We stimulated distant (Fig. 1a,b) or neighboring (Fig. 1c,d) dendritic sites with various delays and the responses were compared to a linear prediction based on summation of single responses (Fig. 1e).

The stimulus protocol was as follows. First, single stimuli were generated with each electrode. Then paired stimuli were produced: electrode 2 (SE2) stimuli occurred 15, 10, 5, 4, 3, 2, 1 and 0.2 ms before electrode 1 (SE1) stimuli, then the order was reversed and SE1 stimuli occurred before SE2 stimuli. The complete series of two single and 16 paired stimuli was repeated 10–30 times, depending on the stability of the recording. Single responses were used to calculate the means and standard errors (SEM) of the linear predicted sums for the paired stimuli by summation of single trial responses with the appropriate time shifts (Fig. 1e).

We would like to note in passing that the 'zero-delay' or synchronous stimulus was replaced by a paired stimulus with a short delay of 200 μs which prevented nonlinearities (supra- or sub-linear summation) observed only in responses to perfectly synchronous stimuli. The nonlinearities were also observed under voltage clamp conditions, suggesting that they were not due to voltage sensitive mechanisms but rather to interactions between the electric fields generated by the two electrodes.

We chose to stimulate the distal dendritic sites (Fig. 1a,b) because it would: (1) maximize the distance the postsynaptic signal had to travel trough the dendrite, thus maximizing the chances of activating possible voltage dependent dendritic conductances; and (2) it would minimize antidromic stimulation of the ascending branches of granular cell axons who form several en-passant synapses with Purkinje cells [5,14].

Since the parallel fibers proper have no collaterals and form only a single en-passant synapse per cell [7], surface stimulation in sagittal cerebellar slices only affects postsynaptic structures in the column directly underneath the effective diameter of the stimulating electrode (i.e. the cathode). Stimulation of parallel fiber input was therefore very local, sometimes probably restricted to a single dendritic branch as indicated by the fact that, at about one third of all stimulation sites, switching the polarity of the stimulating current, which is equivalent to shifting the site of stimulation by about 10 μm, resulted in failure to elicit a postsynaptic response.

Summation of pEPSPs arriving at distant dendritic sites was linear, for all stimulus delays tested (Fig. 2a, left). When stimulating neighboring dendritic sites, however, summation was sub-linear for delays of 10 ms and smaller (Fig. 2a, right). The difference between the linearly predicted and the measured responses increased with decreasing delay between stimuli.

Purkinje cells of young rats are electrotonically compact, allowing the dendritic voltage to be clamped to a specific value by somatic current injection. Voltage dependent activity can thus be controlled experimentally and synaptic currents can be measured [4]. When clamping the voltage to −80 mV synaptic currents summed linearly, independent of temporal delay and spatial distance between inputs (Fig. 2b). The fact that synaptic currents summed linearly under all conditions, supports the assumption the non-linear summation observed under current clamp conditions was voltage dependent and not due to shunting by synaptic conductances. Also, blocking inhibitory synaptic transmission did not alter our results. The data examples shown in Figs. 1 and 2 were measured in the presence of (−)-Bicuculline-methiodide and 2-Hydroxysaclophen (50 μM each) in the bath.

Two possible voltage dependent mechanisms could cause sublinear summation: (1) a reduction in driving force due to the depolarized membrane potential; and (2) the activation or de-activation of voltage gated conductances which would either shunt input or actively hyperpolarize the membrane potential. From our in vitro data we cannot discriminate between those two effects. We therefore compared the electrophysiological data with results obtained from a simulation of the experiment using a passive compartmental model of a Purkinje cell [15,16]. For the generation of atomically realistic compartmental models, Purkinje cells were stained with biocytin [12] and morphologically reconstructed in three dimensions using custom made technology [3]. The digitized neurons consisted of sets of points defined in x, y and z coordinates with a value for the diameter at each point. Passive compartmental models were generated using the Nemosys simulation environment [10]. Stimulation experiments

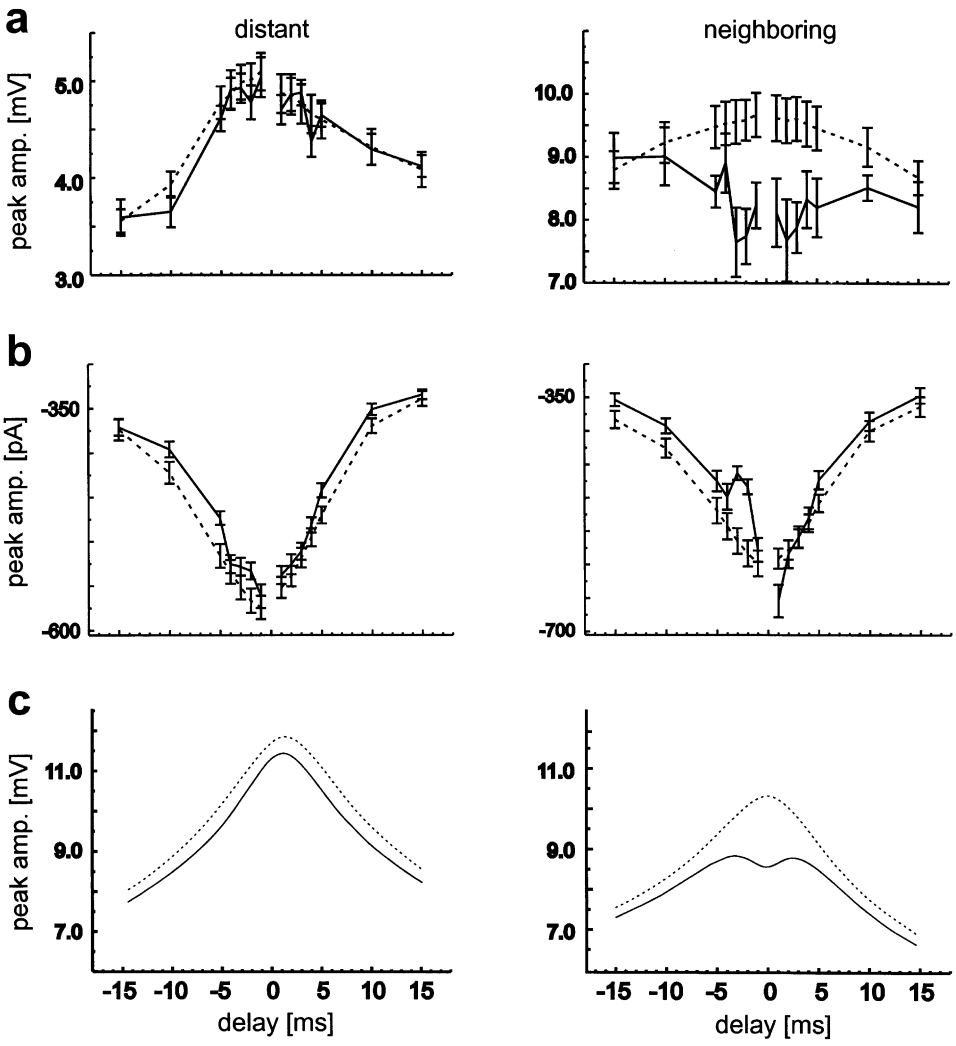

Fig. 2. Measured (solid lines) and predicted (dashed lines) response amplitudes of Purkinje cells in vitro (a,b) and of a model Purkinje cell (c). Comparison of results obtained with stimulation of distant (left column) and neighboring (right column) sites under current clamp conditions in vitro (a), in the model (c) and under voltage clamp conditions in vitro (b). Purkinje cell data in (a) and (b) were recorded in the presence of GABA antagonists in the bath. (a) Mean peak amplitudes of somatic voltage responses to paired stimuli. Summation of pEPSPs arriving at distant dendritic sites was linear independent of input delays. Closely neighboring inputs summed sublinearly when arriving with delays of 10 ms or less from each other. Although the exact shape of the delay-amplitude curve for neighboring inputs varied between cells, the difference between measured response and the linear prediction always increased with decreasing delays. (b) Mean peak amplitudes of excitatory postsynaptic currents recorded in response to stimulation of distant and neighboring dendritic sites. Synaptic currents summed linearly independent on stimulus delay and distance between input sites. (c) Somatic voltage responses of a compartmental model of a Purkinje cell to two simulated inputs at distant and neighboring sites. For better visibility the dashed line is shifted by adding a constant value. Error bars show SEM.

performed in vitro were repeated in the model Purkinje cells by local activation of conductances at distant or neighboring dendritic sites. Model parameters were: $R_{m,\ \mathrm{soma}}$ (Ωcm^2) = 760; $R_{m,\ \mathrm{dendrite}}$ (Ωcm^2) = 45740; R_i (Ωcm) = 225; C_m ($\mu F\ cm\ -\ ^2$) = 1.0; g_{syn} (mS/cm^2) = 1.0; E_{rev} (mV, relative to resting potential) = 140. The time course of synaptic conductance followed an alpha function with a time to peak of 1 ms. Stimulated dendritic area per stimulus was about 3×10^{-6} cm^2. Somatic potentials had a time to peak of ca. 4 ms and a peak amplitude of 5.4 mV. Synaptic input to the model neurons was simulated by activation of conductances on the dendrite. The results from the model study, summarized in Fig. 2c, closely matched the results obtained in current clamp measurements in vitro (Fig. 2a), indicating that a reduced driving force alone can produce the observed integrative properties.

Similar integrative properties have been seen in studies on dendritic integration in fly visual interneurons where sublinear summation of synaptic input occurred when two dendritic areas were stimulated in close vicinity, but not when they were distant from each other [6]. Here again, based on model studies the conclusion was drawn that active membrane properties do not contribute to the observed effect.

As a functional consequence of the Purkinje cell integrative properties in young rats the effect of synchronized parallel fiber input would strongly depend on the

spatial distribution of inputs. Spatial extend of dendritic depolarization, however, and hence the electrical independence of inputs, depends on the electrotonic compactness of the dendrite. This, in turn, is not constant but changes in dependence on background synaptic activity [2,9,11]. With increasing background activity dendritic conductance is increased and with it the electrotonic length of the dendrite. One may speculate that 'neighboring' synapses showing sublinear integration at low background activity may sum linearly with increased synaptic bombardment.

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References

[1] B. Antkowiak, D. Heck, Effects of the volatile anaesthetic enflurane on spontaneous discharge rate and $GABA_A$-mediated inhibition of Purkinje cells in rat cerebellar slices, J. Neurophysiol. 77 (1997) 2525–2538.
[2] Ö. Bernander, R.J. Douglas, K.A.C. Martin, C. Koch, Synaptic background activity influences spatiotemporal integration in single pyramidal cells, Proc. Natl. Acad. Sci. USA 88 (1991) 11569–11573.
[3] A. Borst, J. Haag, The intrinsic electrophysiological characteristics of fly lobula plate tangential cells: I, passive membrane properties, J. Comput. Neurosci. 3 (1996) 313–336.
[4] A. Borst, D. Heck, M. Thomann, Voltage signals of individual Purkinje cell dendrites in rat cerebellar slices, Neurosci. Lett. 238 (1997) 29–32.
[5] G. Gundappa-Sulur, E. DeSchutter, J.M. Bower, Ascending granule cell axon: an important component of cerebellar cortical circuitry, J. Comp. Neurol. 408 (1999) 580–596.
[6] J. Haag, M. Egelhaaf, A. Borst, Dendritic integration of motion information in visual interneurons in the blowfly, Neurosci. Lett. 140 (1992) 173–176.
[7] R.J. Harvey, R.M.A. Napper, Quantitative studies on the mammalian cerebellum, Prog. Neurobiol. 36 (1991) 437–463.
[8] M. Hausser, N. Spruston, G.J. Stuart, Diversity and dynamics of dendritic signaling, Science 290 (2000) 739–744.
[9] D. Heck, J.F. Léger, E.A. Stern, A. Aertsen, Size and summation of synchronous and asynchronous population PSPs in rat neo-cortical neurons: intracellular recording in vivo with dual intracortical microstimulation, Soc. Neurosci. Abstr. 26 (2000) 6096.
[10] M.L. Hines, N.T. Carnevale, The NEURON simulation environment, Neural Comput. 9 (1997) 1179–1209.
[11] W.R. Holmes, C.D. Woody, Effects of uniform and non-uniform synaptic 'activation-distributions' on the cable properties of modeled cortical pyramidal neurons, Brain Res. 505 (1989) 12–22.
[12] K. Horikawa, W.E. Armstrong, A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates, J. Neurosci. 25 (1988) 1–11.
[13] J.C. Magee, Dendritic integration of excitatory synaptic input, Nat. Rev. Neurosci. 1 (2000) 181–190.
[14] C. Pichitpornchai, J.A. Rawson, S. Rees, Morphology of parallel fibres in the cerebellar cortex of the rat: an experimental light and electron microscopic study with biocytin, J. Comp. Neurol. 342 (1994) 206–220.
[15] W. Rall, Theoretical significance of dendritic trees of neuronal input-output relations, in: R.F. Reiss (Ed.), Neural Theory and Modeling, Stanford University Press, Palo Alto, 1964.
[16] M. Rapp, I. Segev, Y. Yarom, Physiology, morphology and detailed passive models of guinea- pig cerebellar Purkinje cells, J. Physiol. (London) 474 (1994) 101–118.
[17] A. Reyes, Influence of dendritic conductances on the input-output properties of neurons, Ann. Rev. Neurosci. 24 (2001) 653–675.
[18] G.J. Stuart, H.-U. Dodt, B. Sakmann, Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy, Pfluegers Arch. 423 (1993) 511–518.
[19] R. Yuste, D.W. Tank, Dendritic integration in mammalian neurons, a century after Cajal, Neuron 16 (1996) 701–716.